NANOSTRUCTURING OF POLYHYDROXYBUTYRATE INDUCED BY PLASMA EXPOSURE

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Abstract

Biopolymers such as polyhydroxybutyrate (PHB) occupy an important place in regenerative medicine and pharmacy and appropriate material treatment may contribute to application improvement and expansion. We have modified PHB foils by plasma treatment for the purposes of tissue engineering. The substrates were treated by high power plasma under different conditions (plasma power, time exposure and working gas). The applied plasma power was from 50 to 200 W and as a working gas argon and oxygen was employed. Another important part of the work was the investigation of surface changes caused by the plasma treatment. The changes of morphology were studied by AFM and SEM, and also the roughness and the contact angle were determined. It was observed that interesting formations with spongy structure visible on SEM scans were created by high power plasma modification. The treatment caused also the significant increase of roughness. The chemical analyses were performed by FTIR for the detection of new functional groups creation and XPS for the detection of surface element concentration.

Keywords: Polyhydroxybutyrate, plasma, nanostructuring, morphology, surface

1. INTRODUCTION

Plasma is an excellent and cheap tool of surface modification for a wide range of materials which can be applied for various purposes. Plasma can replace numerous conventional wet-chemical methods in high-tech laboratories and industries, with a huge impact in renewable energy, environmental protection, biomedical applications, nanotechnology, microelectronics, and other fields [1]. It may be used from the substrate cleaning [2] or adhesion adjustment [3-5] to the actual modification or surface structuring [6]. Plasma treatment can modify the surface properties of the material without any changes of the bulk [7-9]. The final effect of the modification can be adjusted by setting of working atmosphere, plasma power and pressure or time of exposure [10].

Polyhydroxybutyrate (PHB) is a resorbable biomaterial what makes it a very good candidate for healthcare applications. It is widely used in a form of nanoparticles for drug delivery system [11, 12] or in orthopedics applications for bone and cartilage substitution and repair in a form of sutures, rods, screws, pins and plates [13, 14] or supported scaffold for tissue engineering [13]. PHB is also commercially used as a packaging material. Polyhydroxybutyrate belongs to the family of polyhydroxyalkanoates, linear polyesters produced by bacterial fermentation. PHB homopolymers are highly crystalline, extremely fragile, and degrade near its melting point. These properties complicate the manufacturing process and the use of product; therefore, it is usually produced as a copolymer of PHB with polyhydroxyvalerate (PHV) [15].

In this paper, we have used the interaction of plasma treatment with foils of polyhydroxybutyrate for new surface structures creation. The changes caused by plasma modification were investigated by various analytical methods. For morphological observation and roughness measurement were employed AFM and SEM, chemical composition of surface was determined by XPS, changes in functional groups were detected by FTIR, and also goniometry was employed to obtain information of wettability.

2. EXPERIMENTAL: MATERIAL, MODIFICATION AND CHARACTERIZATION

For experiments we used polymer foils of polyhydroxybutyrate (PHB) with 8% polyhydroxyvalerate (thickness 50 µm, density 1.25 g cm^{-3}, supplied by Goodfellow Ltd., Cambridge). The foils were modified in argon (20 sccm) or in oxygen/argon plasma (20/10 sccm) (OXFORD instruments, Plasmalab80Plus) for 240 s, by plasma power of 50 or 200 W under pressure of 150 mTorr.

For characterization we used Contact angle measurement, Atomic force microscopy (AFM), Scanning Electron Microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and Fourier transform infrared spectroscopy (FTIR). The contact angles of water were measured next day after treatment at room temperature at least at six positions using a drop shape analyzer (DSA 100, KRÜSS GmbH, DE). Drops of distilled water of the volume of 2.0 ± 0.2 µl were deposited on the tested samples and evaluated by the ADVANCE System. The error of contact angle measurement was below 10 %. AFM (instrument VEECO CP II in tapping mode and a Si probe RTESPA-CP with the spring constant 20-80 N m^{-1}) and SEM (FIB-SEM, LYRA3 GMU, Tescan, Czech Republic) were employed for surface morphology investigation and AFM was also used for roughness measurement. For visualization, necessary for SEM characterization of the samples, the conductive Au layer was applied by sputtering technique (BAL-TEC SCD 050 equipment). The chemical changes of the PHB surface layer were studied by XPS (Omicron Nanotechnology ESCAProbeP spectrometer with X-ray source monochromatic at 1486.7 eV and CASA XPS program for evaluation). Changes in the surface chemical structure of polymer samples were examined by FTIR using Bruker ISF 66/V spectrometer NICOLET 6700 (Thermo, Nicolet, USA) with diamond ATR extension GladiATR. The difference FTIR spectra, which are discussed in this work, were determined by subtracting FTIR spectra of pristine samples from those of plasma treated ones.

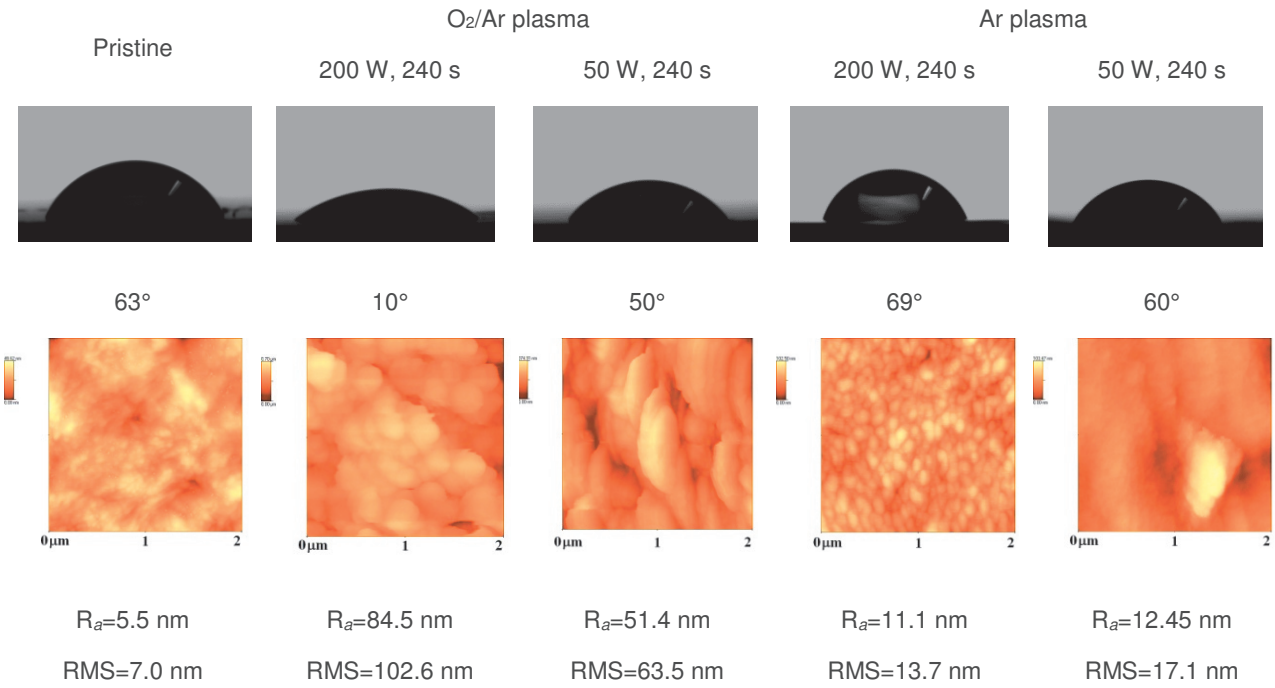

Figure 1 The pictures and the values of water contact angle of pristine and plasma treated samples by different conditions, which are introduced in the figure on the top. In the lower part of the figure are shown 2D AFM scans (2 × 2 µm) and their values of roughness (R_a is the arithmetic mean of surface roughness and RMS is the root mean square roughness; both in nm)

3. RESULTS AND DISCUSSION

For initial information of material changes caused by plasma treatment we used contact angle measurement of water drop. Various values of contact angles of different samples indicate changes in properties such as morphology, roughness and surface chemistry. The information about contact angle measurement, as well as values of roughness and AFM scans illustrating surface topography, are available in **Figure 1**. As it was suspected, in general, the higher plasma power (200 W) has stronger influence on wettability changes in comparison with pristine substrate and resulted in a more significant topography changes than lower plasma power (50 W). Even more interesting parameter than plasma power is content of plasma atmosphere. Presence of oxygen in argon atmosphere has increased etching and oxidation effects, leading to a deepening of formed structures accompanied by a significant increase of roughness. The most interesting effect of plasma treatment was observed on PHB substrate exposed for 240 s with 200 W in O_2/Ar atmosphere. This modification have caused great improvement of wettability; the contact angle was reduced to 10° from original 63°, when the measurement was performed immediately after a drop application, but the value was not stable and has decreased with prolonged time of measurement after the drop placement. The effect of decreasing value with prolonged time of measurement was observed only for these parameters because all other values were stable in time. The biggest increase of roughness (to 84.5 nm for R_a and to 102.6 nm for RMS in comparison with non-modified PHB with value of roughness R_a=5.5 nm and RMS=7.0 nm) and most interesting structure also belongs to the substrate exposed for 240 s with 200 W in O_2/Ar atmosphere. This structure is better demonstrated by SEM images in **Figure 2**, where it is also possible to observe the “mushroom-like” formations on scans with sample tilting of 54.8°. For comparison the SEM images of PHB pristine and plasma treated sample with 200 W for 240 s in Ar atmosphere are added.

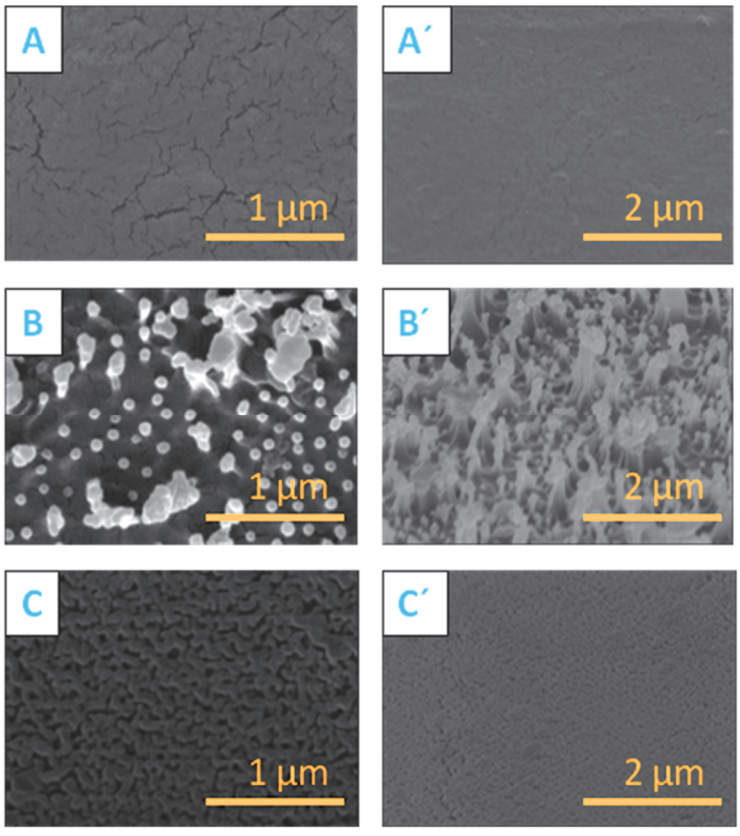

Figure 2 2D SEM images of PHB pristine (A) and samples treated by plasma (200 W and 240 s) in O_2/Ar atmosphere (B) and in Ar atmosphere (C). Right images (A´,B´,C´) represent also 2D SEM images but after tilting 54.8°

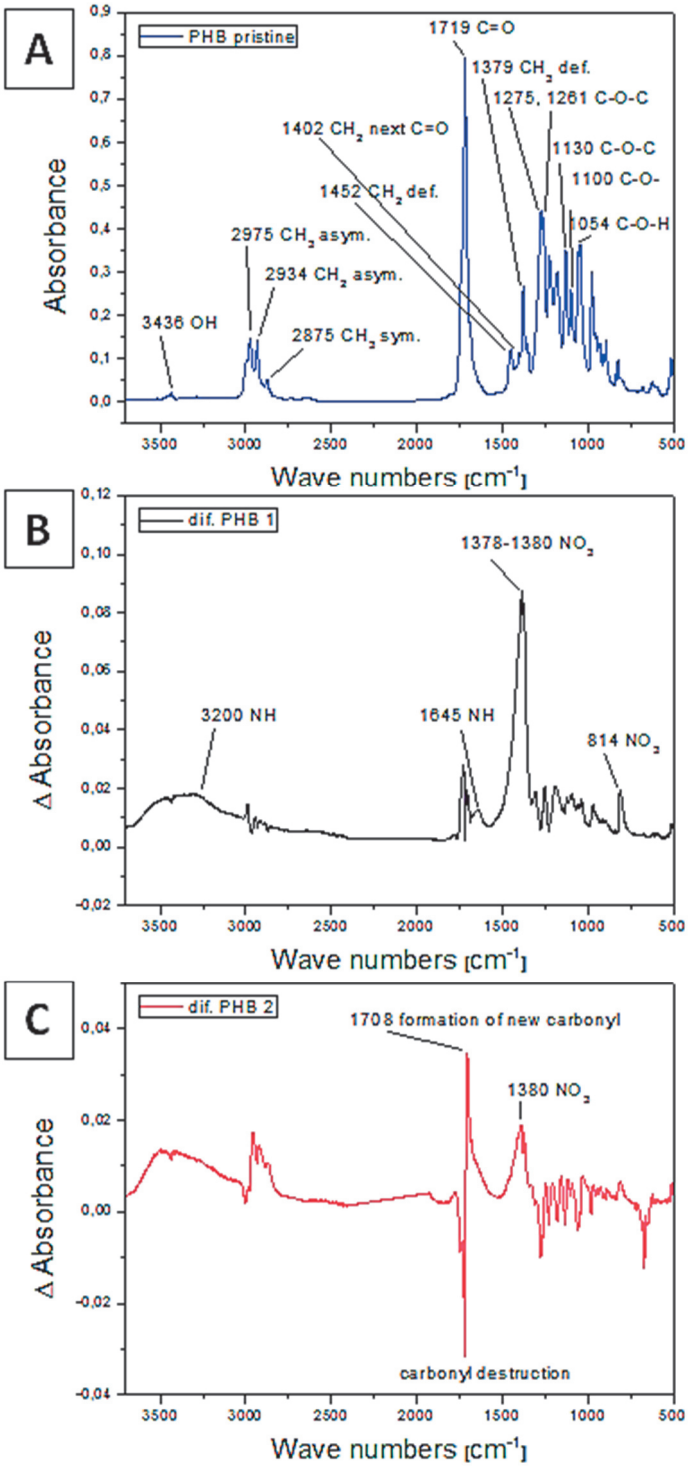

Figure 3 FTIR spectrum of PHB pristine (A) and differential spectra of PHB treated with 200 W and 240 s in O_2/Ar atmosphere (B) and in Ar atmosphere (C)

Plasma modification affects also the surface chemistry of substrates. **Table 1** describes the changes in element concentration by XPS for pristine and treated samples under conditions introduced in the table. Non-modified polymer PHB contains carbon, oxygen and hydrogen (which is not detectable by XPS method). During the modification process the oxygen concentration has decreased (pristine PHB contains 33.32 at.% of oxygen) in favor of carbon for all samples. The biggest decrease has occurred during the treatment for 240

s with 200 W in O_2/Ar atmosphere, although oxygen was present in the working gas. The presence of oxygen in working gas exhibits the etching effects in a way that some of the oxygen functional groups have been ablated from the surface of the substrate. On, contrary the nitrogen was incorporated into the structure, although it was not included in the working gas. Nitrogen was incorporated into the material immediately after the modification, when the formed radicals have reacted with the ambient atmosphere.

The FTIR with ATR crystal can provide results in depth up to 200 nm, i.e. much deeper than detection limit of XPS, still the results from FTIR corresponds with the results from XPS. From differential spectra we got information about the newly created functional groups and also about the functional groups diminishing. **Figure 3a** shows the spectrum of PHB pristine and **Figure 3b** and **C** represents the differential spectra of modified samples treated with 200 W for 240 s in O_2/Ar atmosphere or just Ar atmosphere. The formation of nitrogen groups NH (peaks location at 1645 and 3200 cm^{-1}) and N-O_x (peaks location at 1378-1380 and 814 cm^{-1}) was proved after modification in the O_2/Ar atmosphere. After modification in "pure" Ar atmosphere, no NH groups were observed but formation of new carbonyl has been reported (peaks location at 1708 cm^{-1}).

Table 1 Element concentration of carbon, oxygen and nitrogen in the surface layer measured by XPS method for pristine and plasma treated samples by different conditions

Sample		C [at%]	O [at%]	N [at%]
Pristine		66.68	33.32	0.00
O_2/Ar plasma	200 W, 240 s	66.30	29.98	3.72
	50 W, 240 s	67.02	30.18	2.80
Ar plasma	200 W, 240 s	78.49	21.51	0.00
	50 W, 240 s	73.92	24.70	1.39

4. CONCLUSION

By plasma modification were prepared samples of PHB with various structures, depending on the applied parameters. The modification caused changes of wettability, structure, roughness as well as surface chemistry. According to the assumption, at higher power has provided more interesting results and topographies. The most interesting "mushroom-like" structure was prepared by plasma power 200 W in O_2/Ar atmosphere. Treatment in all cases has lead to a decrease of surface atomic concentration of oxygen.

ACKNOWLEDGEMENTS

This work has been supported by the Ministry of Health of CR under the project 15-33018A and Grant Agency of the Czech Republic under the project 13-06609S.

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